Recent Developments in *Hydrangea* Interspecific Hybridization

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Significance to Industry: Hydrangeas are among the most popular flowering shrubs with annual U.S. sales of approximately $32 million. While several species are cultivated and many cultivars are available, little work has been done on combining the best characteristics from different members of this genus via interspecific hybridization. This report describes the production of a hybrid between *H. arborescens*, which is one of the most cold-hardy members of the genus, and *H. involucrata*, which produces lavender-blue flowers. Production of this hybrid represents the first step in combining cold hardiness and flower color in *Hydrangea*. This work may eventually lead to the development of superior cultivars and expanded sales of *Hydrangea* in colder areas of the U.S.

Nature of Work: *Hydrangea arborescens*, or smooth hydrangea, offers large corymbs of pure white flowers in early summer and is cold-hardy to zone 4. *Hydrangea involucrata* produces lavender-blue flowers in mid-summer, but is only rated as cold-hardy to zone 6 or 7. The objective of this study was to hybridize these two species with the intention of combining the cold hardiness of *H. arborescens* with the flower color of *H. involucrata*.

Reciprocal crosses were made between *H. involucrata* and *H. arborescens* 'Dardom' (White Dome®) during Summer 2003 using previously published techniques (3). Seeds were collected in early fall and sown in a greenhouse in January 2004. All progeny of the interspecific crosses were grown in a greenhouse following germination.

RAPD markers were used to verify hybridity. Young expanding leaves were collected from six of the progeny, freeze-dried, and stored at -70° C (-94° F) until needed. DNA was extracted with a Dneasy Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. DNA content was estimated by loading samples into a 1% agarose gel and comparing visually with known standards. Amplification reactions were carried out in 25µl volumes containing 1X MasterMix (Eppendorf AG, Hamburg, Germany), 0.2 µM primer set 100/4 (UBC, Vancouver, British Columbia, Canada), and 10 ng DNA template. Amplification involved an initial denaturation step of 95°C (205 °F) for 5 min and then 45 cycles of 95 °C for 1 min, 36 °C (97 °F) for 1 min and 72 °C (162 °F) for 2 min. This was followed by a final extension step of 72 °C (162 °F) for 2 min. After amplification, 2 µl of each sample was loaded onto a 1% agarose gel and run in 1X TBE buffer at 120V for 35 min. Gels were stained with ethidium bromide and reaction products were viewed using an AlphaImager (Alpha Innotech Corp., Alameda, CA).

Results and Discussion: Only crosses made with *H. arborescens* as the maternal parent resulted in viable seed production (Table 1). Of the more than 500 seed obtained, only eight plants remain alive. The surviving plants possess a range of growth rates, currently ranging from 8 to 35 cm (3.1 to 13.5 in) in height and from 12 to 50 cm (4.7 to 19.5 in) in width. Leaf shape is consistent among progeny and is intermediate in appearance between parents.

A total of 43 primers were tested, generating from 1 to 6 markers. The size of the amplified DNA fragments ranged from 100 to 1200 base pairs. The progeny had a combination of both parental species banding patterns in four primers (UBC: 335, 336, 341, 349; Fig.1). Six primers produced banding patterns identical in the hybrids and paternal parents (UBC: 308, 337, 338, 345, 348, 349). Banding profiles generated from these ten primers confirmed hybridity in the progeny tested.

Previous efforts to combine flower color and cold hardiness in *Hydrangea* via interspecific hybridization have met with only limited success. *Hydrangea macrophylla* × *H. paniculata* (5) hybrids could be produced only by using in vitro embryo rescue. These hybrids were lacking in vigor and sterile (4). Production of *H. macrophylla* × *H. arborescens* hybrids required both embryo rescue and subsequent regeneration from callus culture (1); all plants obtained had aneuploid chromosome numbers (2). This report details the first confirmed *Hydrangea* interspecific hybrid that was obtained without the use of embryo rescue and that involved *H. involucrata* as one of the parents. We will continue to work with this hybrid in an effort to develop cold hardy hydrangeas with blue flower coloration.

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Table 1. Results of hybridization between *H. involucrata* and *H. arborescens* 'Dardom'.

cross	no. crosses made	no. viable seed obtained	no. surviving plants
H. involucrata × *H. arborescens* 'Dardom'	78	0	- - -
H. arborescens 'Dardom' × *H. involucrata*	74	36	8

Figure 1. RAPD profiles generated from primer UBC-341. Lane 1 = 100 kb marker, HA = *H. arborescens* 'Dardom', 2-13 = *H. arborescens* 'Dardom' × *H. involucrata* progeny #735-2 through 735-13, HI = *H. involucrata*. The maternal and paternal parents had only one band each: *H. arborescens* was represented with a band of 600 bp and *H. involucrata* with a 400 bp band. Both bands were present in all progeny tested.